Glucose Starvation Blocks Translation at Multiple Levels

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Glucose starvation blocks translation at multiple levels

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Deficiency of glucose, even under sufficient amino acid supply, turns off translation, and promotes catabolic processes to aid cell survival. A recent report by Yoon et al. (2019) shows that glucose is required for the full activity of the leucyl-tRNA synthetase LARS1, and maintains mTORC1 function via LARS1 to enhance translation. Glucose starvation abolishes both effects via phosphorylation of LARS1 by the AMPK-ULK1 signaling pathway. This study supports the idea that glucose starvation inhibits translation at multiple levels.

Translation is an energy-consuming process that consumes at least two molecules of ATP and two of GTP to add each new amino acid to the growing polypeptide chain.

Cells employ multiple regulatory mechanisms to ensure that this energy-consuming anabolic process can only take place when ample nutrients and growth factors are available. Availability of glucose, the major energy source for most cells, is known to be a prerequisite for translation. It has long been known that AMP-activated protein kinase (AMPK), a cellular sensor of energy and nutrients, is activated upon glucose starvation, in turn switching off the mammalian target of rapamycin complex 1 (mTORC1) pathway, which is critical for both the initiation and elongation steps of translation (Johanns et al., 2017). AMPK inhibits mTORC1 by dual mechanisms: i) phosphorylation of tuberous sclerosis 2 (TSC2) (Inoki et al., 2003), leading to inhibition of RHEB (Ras homolog enriched in brain), a lysosome-localized small GTPase that facilitates mTORC1 activation only in the GTP-bound status; ii) phosphorylation of RAPTOR (regulatory-associated protein of mTOR), a component of the mTORC1 complex (Gwinn et al., 2008). As well as these effects via mTORC1, AMPK also phosphorylates and activates eukaryotic elongation factor-2 (eEF2) kinase, which then triggers phosphorylation and inactivation of eEF2 itself to directly block the elongation step of translation (Johanns et al., 2017).

The recent report by Yoon et al. (2019) adds a new tier to the regulation of translation by AMPK in response to glucose availability. They first reported a new mechanism in which leucyl-tRNA synthetase 1 (LARS1), which catalyzes the ATP-dependent ligation of L-leucine to its cognate tRNA, can accelerate the inhibition of mTORC1 in low glucose in an AMPK-dependent manner. LARS1 has been previously identified as a leucine sensor that binds to RAGD, a

lysosome-localized Rag GTPase essential for the stimulation of mTORC1 by amino acids, thus promoting conversion of RAGD from the GTP- to the GDP-bound state (Han et al., 2012). RAGD:GDP subsequently promotes mTORC1 translocation to the lysosomal surface to allow its activation by leucine. Intriguingly, the affinity of LARS1 towards RagD, as well as its ability to activate mTORC1, was reduced in low glucose even in the presence of leucine, indicating that glucose may exert a dominant regulation of LARS1. Through RNAi screening, they identified AMPK and its substrate ULK1 (Unc-51 like autophagy activating kinase), as being involved in the suppression of the LARS-RagD pathway in low glucose. Mechanistically, ULK1, which is phosphorylated and activated by AMPK in low glucose (Egan et al., 2011; Kim et al., 2011), phosphorylates S391 and S720 on LARS1, reducing its affinity towards RagD. Yoon et al. (2019) also showed that phosphorylation of LARS1 by ULK1 reduces the binding of both ATP and leucine to LARS1, leading to an inhibition of leucine loading onto tRNA and thus limiting protein elongation. This finding introduces the additional concept that glucose starvation directly disrupts an early step in translation, i.e., aminoacylation of tRNA. As a result, increased free intracellular leucine (originating both from enhanced autophagy after AMPK activation/mTORC1 inhibition and reduced translation), can then be used as an alternative carbon source for the catabolic production of ATP (via the TCA cycle) in low glucose. Consistent with this, removal of leucine from glucose-free medium reduced ATP concentrations in rhabdomyosarcoma cells. This study reveals a finely tuned network that connects AMPK to LARS1, as well as mTORC1, to determine the

67 catabolic or anabolic fate of leucine when availability of glucose is limiting.

68 Highly relevant to these findings are earlier reports (Zhang et al., 2017; Zhang et
69 al., 2014) showing that glucose starvation simultaneously switches on AMPK and
70 switches off mTORC1 on the lysosomal surface. In low glucose, decreased levels of
71 the glycolytic intermediate fructose-1,6-bisphosphate (FBP) are sensed by the
72 glycolytic enzyme aldolase, which cleaves FBP into phosphotrioses. Aldolase that is
73 not occupied by FBP inhibits the vacuolar-type H^+-ATPase (v-ATPase) on the
74 lysosomal surface, leading to a change in its interaction with the pentameric Ragulator
75 complex. Under these circumstances AXIN (either AXIN1 or AXIN2), in complex
76 with LKB1, docks onto the v-ATPase-Ragulator complex, triggering phosphorylation
77 and activation of the lysosomal pool of AMPK by LKB1. The translocation of AXIN
78 to lysosomal surfaces also facilitates release of mTORC1 from the lysosome, thereby
79 at the same time inhibiting mTORC1. Consistent with this, when AXIN1 is depleted
80 in MEFs (in which AXIN2 is not expressed), mTORC1 inhibition becomes much
81 slower after glucose removal. AXIN1 exerts this effect by facilitating the GAP
82 activity of the Ragulator complex towards the RagC GTPase. Therefore, we would
83 argue that high glucose does not activate mTORC1, rather it prevents inhibition of
84 mTORC1 via its dissociation from the lysosome. It is important to stress that glucose
85 starvation can still render mTORC1 inactive even in AMPK-null cells, albeit at a
86 slower rate (Zhang et al., 2014). In support for a critical effect of AXIN on the GAP
87 activity of Ragulator, a “constitutively active” RAG, i.e., the GTP-constitutive
88 binding mutant of RAGA or RAGB, completely blocks the inhibition of mTORC1 in

low glucose, despite intact AMPK activation (Efeyan et al., 2013; Zhang et al., 2014). Therefore, the AXIN-dependent and AMPK-independent mechanisms can operate in parallel to control the activity of mTORC1 (Figure 1). It would be interesting in future to compare the dynamics of translational inhibition following knockout of the various components of the AXIN-AMPK-ULK1 pathway. In summary, it is remarkable that glucose, as the most abundant cellular nutrient, exerts so many regulatory roles to control translation.

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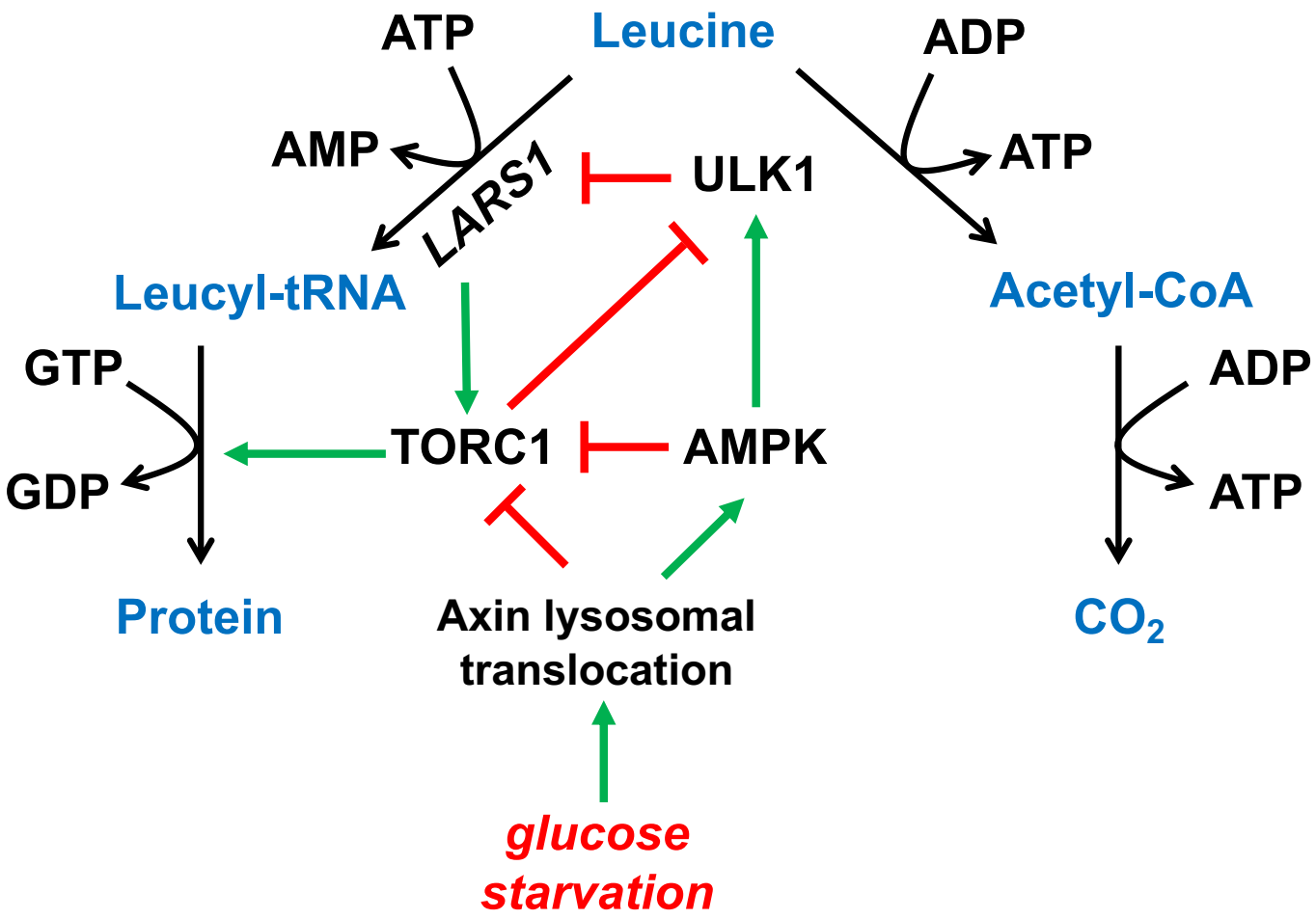
132 and anabolism. Cell Metab. *20*, 526-540.
133

Figure legend

Figure 1. **Glucose starvation inhibits translation at multiple levels to maintain energy balance.**

Upon glucose starvation, AXIN in complex with LKB1 translocates to the lysosomal surface, activating the lysosomal pool of AMPK, which in turn inhibits mTORC1 by phosphorylating TSC2 and RAPTOR. Importantly, the translocation of AXIN itself to the lysosomal surface results in inhibition of mTORC1 by facilitating dissociation of mTORC1 from the lysosome, even in the absence of AMPK. The recent report (Yoon et al., 2019) shows that glucose deprivation blocks translation at an early step of aminocylation. They found that LARS1 is phosphorylated by the AMPK-ULK1 pathway under glucose starvation, promoting conversion of RAGD from the GDP- to the GTP-bound state to inhibit mTORC1, but also impairing the binding capability of both ATP and leucine to LARS1 to block the leucylation of tRNA(Leu). As a result, intracellular levels of free leucine increase due to lesser usage for translation or to increased autophagy. Leucine can thus be used instead as an alternative carbon source for the catabolic production of ATP (right-hand side), while the energy-consuming process of translation is blocked at multiple levels.

Figure 1


ATP
Leucine
ADP
AMP
LARS1
ULK1
ATP
Leucyl-tRNA
Acetyl-CoA
GTP
ADP
TORC1
AMPK
GDP
ATP
Protein
Axin lysosomal translocation
CO_2
glucose starvation